THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 67:26–30, 1976
Vol. 67, No. 1

Printed in U.S.A.

CYTOKINETICS AND CHEMOTHERAPY OF PSORIASIS

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The successful treatment of psoriasis with folic acid antagonists during the past 25 years has led to extensive research in the areas of cytokinetics and chemotherapy. In this paper we shall review selected aspects of these topics relevant to the treatment of psoriasis. The effectiveness of methotrexate treatment of psoriasis can be related to both the hyperproliferative cytokinetics of psoriasis and an increased biochemical sensitivity of psoriatic epidermal cells to this drug.

Future research goals in chemotherapy of psoriasis include (a) optimizing drug schedules for available drugs; (b) identifying other susceptible biochemical points of selective drug attack; (c) identifying secondary advantages in order to facilitate selective drug action in psoriasis, such as ultraviolet light therapy in combination with a systemic drug; and (d) developing topically effective chemotherapeutic agents. Approaches to research on topical therapy are reviewed with specific reference to animal testing models for psoriasis and percutaneous penetration of topically applied agents.

The story of the chemotherapy of psoriasis began a quarter of a century ago with the first reported use of aminopterin for psoriasis [1]. Subsequent reports describing various aspects of treatment with aminopterin and its successor, methotrexate (MTX), have detailed the growing effectiveness of different drug schedules as well as concerns with toxicity. MTX has remained the principal form of systemic chemotherapy of severe psoriasis.

As a by-product of the success of MTX therapy, research on the basic biologic and biochemical properties of psoriasis has been greatly stimulated. Psoriatic epidermis was noted to have an extremely high number of mitoses [2] and a rapid turnover using isotopic measuring techniques [3, 4]. The 2-day transit time of cells through the viable psoriatic differentiated cell compartment—compared to an approximate 2-week period in normal skin [4]—suggested a faster rate of cell reproduction in the psoriatic basal (proliferative cell) layers. Autoradiographic techniques determined the psoriatic cell cycle to be 37.5 hr, compared to 457 hr for normal cells [5].

Recent interest in the cytokinetics of psoriasis has focused on the physiologic controls of cell proliferation and on application to chemotherapy. The current theories concerning cyclic AMP–GMP control of cell division relate directly to the hyperproliferative state of psoriatic epidermal cells. Experiments with human epidermal cell proliferation have shown that the cell cycle kinetics of normal and psoriatic skin grown in vitro become similar [6]; the normal epidermal cell kinetics speeds up to equal that of the psoriatic cell. What, then, is the true native state of epidermal cells? Is the cell stimulated to proliferate rapidly in psoriasis or inhibited by controls in normal epidermis to proliferate slowly? The obvious interplay of biochemical signals regulating cell proliferation is incompletely understood.

Most cancer chemotherapeutic drugs either act on cells in a specific phase of the cell cycle or affect cells regardless of their temporal location in the cycle (nonspecific cell cycle drugs). Such alkylating agents as nitrogen mustard are among the latter. MTX, hydroxyurea, and cytosine arabinoside are examples of S (DNA synthesis) phase cycle-specific drugs. These drugs affect cells only when they are in the S phase portion of the cycle. Similarly, colchicine and vincristine are specific cell cycle poisons for the M (mitotic) phase. This information can be used to program chemotherapy schedules using one drug or a combination for selected diseases based on their cytokinetics. In this manner a pharmacologically rational schedule for psoriasis was developed utilizing the cell cycle value of 37 hr and an S phase-specific drug, MTX [7]. Administration of three doses of the drug at 12-hr intervals exposes all psoriatic cells to the toxic effects of MTX as they pass through the S phase. The triple-dose therapy schedule, requiring a smaller dose of MTX per week than did previous schedules, is now used for over 60% of MTX-treated patients [8].

This work was supported in part by U. S. Public Health Service Grant AM 14887 07 from the National Institutes of Health, the Comprehensive Cancer Center of Greater Miami N.C.I. Grant CA 14395 03, and the Dermatology Foundation of Miami.

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Abbreviations:
EFA: essential fatty acids
MTX: methotrexate

The vast clinical experience in this area shows that careful use of MTX to clear psoriasis has little effect on other proliferative tissues; normal skin, hair, and nail growth are not significantly altered, and bone marrow elements, the gastrointestinal tract, and buccal mucosa are subject to minimal side effects at relatively higher doses of MTX. There are two current explanations for the selective advantage of psoriasis over other normal proliferating tissues:

1. Cell cycle kinetics advantage. Twenty-three percent of psoriatic epidermal cells are in the S phase at any moment compared to only 4% in normal skin. Thus, a single dose of MTX acting on S phase cells will, at a given time, affect 6 times as many psoriatic cells as normal proliferating epidermal cells. If the dose is continued over 36 hr, 100% of the psoriatic cells will be affected whereas only about 9% of normal cells will enter the susceptible S phase during this time period (4% new S phase cells in normal epidermis every 16 hr). Thus, for tissues proliferating more slowly than psoriatic epidermis, i.e., normal skin, bone marrow, and nail, a differential pharmacologic effect can be readily appreciated.

2. Differential biochemical sensitivity of psoriasis. The inhibition of DNA synthesis in psoriatic epidermal cells is quantitatively greater than that in normal skin for given doses of MTX [9]. Psoriatic cells also demonstrate a selective sensitivity to MTX as measured by mitotic activity and the production of "damaged cells" [10]. Explanation of this selective sensitivity to MTX is still unknown and is discussed elsewhere [11].

CHEMOTHERAPY OF PSORIASIS

Systemic therapy of severe psoriasis with various drugs has been evaluated in many studies. The only drug besides MTX approved by the FDA for treatment of psoriasis is azaribine. Other drugs frequently used or being tested include hydroxyurea and mycophenolic acid, but because of their side effects these drugs are unacceptable.

Research goals for the chemotherapy of psoriasis include the following:

1. Development of optimal schedules for the available systemic drugs. Whereas much valuable experience has been gained by changing MTX dosage schedules, few data are available on different schedules for hydroxyurea and azaribine relating their specific pharmacology to the cellular kinetics of psoriasis.

2. Identification of other susceptible biochemical points for drug attack that will be selective for psoriasis while minimizing toxicity in normal tissues.

3. Identification of drugs for selective action on psoriatic tissue because of secondary advantages. Two examples of this are phototherapy with psoralen and ultraviolet light (PUVA), and mycophenolic acid. PUVA therapy is active only in skin (and eyes) by virtue of the limitations of light penetration to other organs of the body. Although the drug is systemically distributed, toxicity due to the combination of light and drug should not be found elsewhere in the body. Existing data suggest that psoralens alone, in the recommended dosages, are without side effects. Mycophenolic acid is an example of a potentially unique drug principle for use in skin diseases. Systemically administered mycophenolic acid is rapidly converted by the liver to an inactive glucuronide, which cannot penetrate cell membranes. Theoretically, only epidermal tissues and others containing a glucuronidase can hydrolyze the conjugated compound back to its free, active form [12].

4. Development of topical chemotherapeutic agents permitting the use of relatively potent cytotoxic drugs with minimal risk of systemic side effects. The remainder of this paper will deal with selected aspects of topical chemotherapy.

Since psoriasis is a hyperproliferative disease which responds to drugs that affect proliferating cell populations, it is reasonable to assume that these drugs are acting directly on the skin rather than at a distant site. Clinically, nitrogen mustard, thiotepa [13], and nitrosourea [14] have been shown to clear psoriasis topically, providing evidence of mechanisms for local therapeutic control. Injected intradermally into psoriatic skin, MTX will produce the same inhibition of DNA synthesis and mitoses that occurs after systemic administration, suggesting that the drug works locally (although such local infusion of MTX does not clear psoriasis). In an experimental animal system, healing of skin wounds can be prevented by topical administration of MTX, presumably by stopping cell proliferation. Regional perfusion of MTX produces cytotoxic effects directly on tumors. For all these reasons, drugs like MTX and other chemotherapeutic agents should act directly on a hyperproliferative disease like psoriasis.

THE ENIGMA OF THE INEFFECTIVENESS OF TOPICAL MTX

Several factors may explain the topical ineffectiveness of MTX: (a) lack of percutaneous penetration; (b) drug inactivation in skin during penetration; (c) a major effect at a distal site; and (d) the necessity for conversion elsewhere into an active compound. Brief discussion of current investigation into these possibilities follows.

(a) Studies show that MTX preparations in an aqueous vehicle produce negligible penetration of the stratum corneum and thus the drug is not delivered to the proliferative epidermal cells where it is thought to act. It is assumed that if the drug penetrates the stratum corneum it will penetrate the epidermal cells. This appears to contribute to the lack of topical effect.

(b) Incubation of MTX with skin shows no evidence of significant drug alteration or inactivation (McCullough and Weinstein, unpublished

observations). Similar findings have been observed when the drug is given to animals or patients systemically.

(c) If MTX acts systemically at a site distant from the psoriatic skin, two mechanisms are suggested. First, psoriasis might be controlled by a different organ susceptible to MTX, such as the liver. Second, MTX might prevent the production of an unknown circulating compound such as a reduced folate coenzyme necessary to maintain the activity of the psoriatic lesion. No evidence is currently available to support these possibilities.

(d) There is no evidence that MTX is converted into a more active metabolite at a distal site.

DEVELOPMENT OF TOPICAL ANTIPSORIATIC DRUGS

A research program to develop topical antipsoriatic drugs has many biochemical, pharmacologic, and clinical experimental aspects. After selecting potentially valuable compounds from biochemical predictive assays, it would be advantageous to test them on experimental animals. Unlike animal leukemias or tumors, an experimental animal model system has never been developed for psoriasis. In recent years, much effort has been invested in creating psoriasis-testing models, several of which will be reviewed here.

The mouse vaginal mitotic screen was developed by Van Scott et al [15]. Vaginal epithelium stimulated by estrogen to hyperproliferation is exposed to drugs by intravaginal application. The vaginal and neighboring rectal epithelia are examined histologically for mitotic counts. Inhibition of mitoses in the vaginal epithelium with no effect on the rectal epithelium indicates a direct local drug effect on the proliferative vaginal cells. A normal mitotic count in the rectum is the simultaneous control ruling out systemic absorption as the cause of the vaginal mitotic inhibition. This simple assay is useful for rapid screening of many drugs.

The mouse vaginal assay has been modified in our laboratory to test the effect of drugs on the inhibition of DNA, RNA, and protein synthesis [16]. After the drugs are introduced intravaginally, the animals are injected intraperitoneally with isotopic precursors, [^{3}H]thymidine or [^{3}H]deoxyuridine for DNA synthesis; [^{3}H]uridine for RNA synthesis; or [^{3}H]leucine for protein synthesis. Vaginal–rectal bloc specimens are examined autoradiographically for incorporation of these precursors as a measure of their respective inhibition by each drug after "local" drug administration.

Another simple assay system studied in our laboratories involves the production of discrete cutaneous wounds with punch biopsies. Healing of these wounds requires epidermal regeneration, which might be halted or retarded by topical application of effective agents. This assay has been used in both human [17] and nonhuman skin to test the local effects of drugs on epidermal regeneration as a model for psoriasis.

An assay by duVivier and Stoughton uses ultraviolet light-stimulated hyperproliferative epidermis from hairless mice [18]. Changes in the hyperproliferative process brought about (effected) by the drugs are measured by mitotic counts, thymidine labeling indices, and/or quantitative measures of DNA synthesis. Topical local effects can be separated from systemic effects.

One model for a psoriasiform disease is the essential fatty acid (EFA)-deficient rat [19]. The proliferative aspects of this model are scaling hyperkeratotic skin with rapid cell proliferation (unpublished observations). Comparison of normal and EFA-deficient rat skin demonstrates a difference in epidermal thickness and respective labeling indices of 4 and 25% (Fig.). The transit time of thymidine-labeled cells from the basal to the granular layer is 2 days. These numbers are comparable to normal and psoriatic skin, suggesting a biologic similarity to the clinical disease.

Another animal model with great potential for studying psoriasis and many other diseases—particularly tumors—is the athymic "nude" mouse, which is able to continue the growth of heterologously transplanted tissue. Krueger et al reported graft survival periods of 2 to 3 months in the first studies of psoriasis transplants in nude mice [20]. At this writing, only histologic criteria have been used to show that the graft still appears as the original human tissue. Briggaman and Weinstein are investigating the cellular kinetics of lamellar

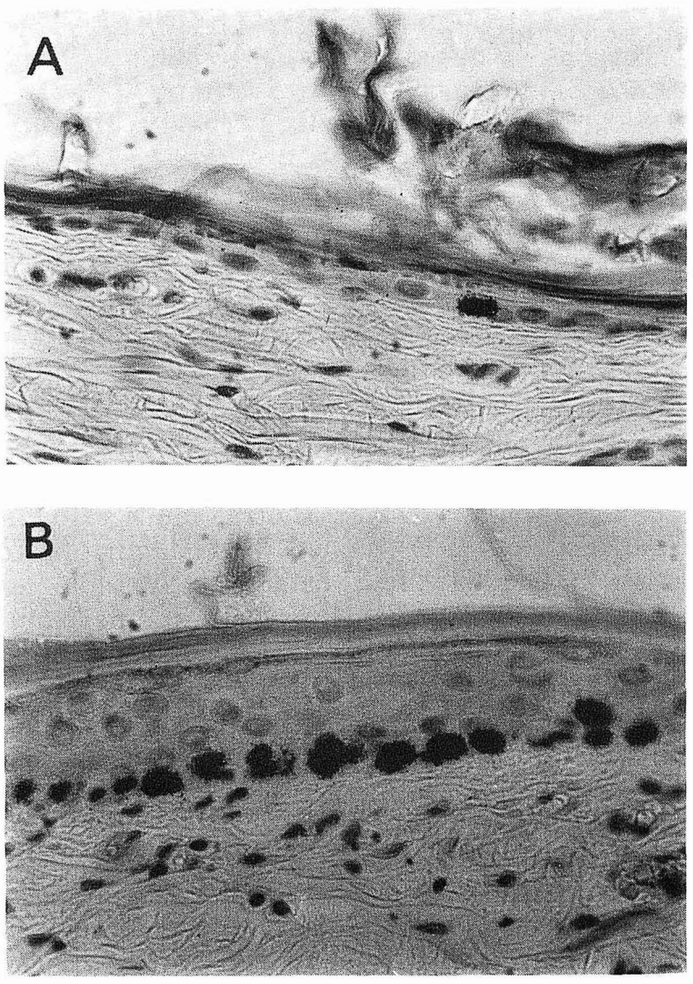

FIG. Autoradiographs of [^{3}H]thymidine labeling of (*A*) normal rat skin, and (*B*) EFA-deficient rat skin (× 160).

ichthyosis in the same animal model. The experimental thymidine transit time in the graft site is similar to that found in patients with lamellar ichthyosis.

PERCUTANEOUS PENETRATION AND TOPICAL THERAPY

Another problem to be considered in the rational development of a topically effective antipsoriatic drug involves percutaneous penetration. The low permeability of the skin acts as a selective barrier to the penetration of some topical medications. Consideration must therefore be given to the inherent ability of a given drug to penetrate this barrier as well as to the selection of the appropriate vehicle to optimize topical penetration. In vitro test systems measuring drug penetration through excised human skin are useful for studying the percutaneous penetration of topical chemotherapeutic agents. We have used this technique to study the factors affecting human percutaneous penetration of MTX and its analogues in vitro [21]. We found no percutaneous penetration of [^{3}H]MTX from an applied aqueous solution (less than 0.005% of the applied drug dose). However, [^{3}H]MTX found in the epidermis was localized to the outermost layers of the stratum corneum. These results suggest that one explanation for the clinical ineffectiveness of topical MTX in psoriasis is the lack of sufficient penetration into or through the viable epidermis. One way to enhance the percutaneous penetration of a drug is to increase the lipophilic character of the molecule. We found that both the epidermal concentration and percutaneous penetration were enhanced by increasing the lipid solubility of MTX through chlorination and/or esterification of the parent molecule (Tab.).

Another approach involves alteration of the vehicle which plays a critical role in the release and partitioning of the drug to the skin surface. In our study, the in vitro percutaneous penetration of MTX was enhanced approximately 140-fold by incorporating it into the vehicle *n*-decylmethylsulfoxide. These investigations of percutaneous penetration will aid in selecting and compounding other potential topical drugs and are essential to the understanding of any lack of topical activity.

TABLE. *Percutaneous penetration of various folic acid antagonists through human skin in vitro*

Drug[a]	Ratio of penetration of drug/MTX [21]		
	Epidermis	Saline	Partition coefficient ratio [21][b]
Methotrexate	1.0	1.0	1
Methotrexate-dimethylester	14.53	1.22	1,150
Dichloromethotrexate	1.14	1.27	10
Dichloromethotrexate-dimethylester	14.87	3.04	1,632

[a] .05% soln of drug applied to epidermal chamber of a glass diffusion cell and incubated for 20 hr at 28°C. Drug concentration in the epidermis and in the lower dermal saline reservoir was determined by dihydrofolate reductase assay.

[b] Ratio of partition coefficient of drug/MTX in *n*-octanol–sodium phosphate buffer.

The empirical approach to drug selection and patient testing has been widely used with varying degrees of success. Compared to the scientific approach, it must bear the burden of local and federal regulatory agencies governing the criteria for human testing. There is now a strong effort to combine the scientific and empirical approaches in screening topical chemotherapeutic drugs for treatment of psoriasis. The National Institute of Arthritis, Metabolism, and Digestive Diseases has recently awarded a contract to the University of Miami Department of Dermatology to organize a multi-institutional study of selected chemotherapeutic agents for the topical therapy of psoriasis, a clinical study that may lead to a better understanding of safe and effective topical treatment.

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